SYSTEMIC AND PULMONARY INFLAMMATORY RESPONSE ASSOCIATED WITH OPEN HEART SURGERY INVOLVING CARDIO-PULMONARY BYPASS; CHANGES IN CYTOKINE BALANCE AND PULMONARY FUNCTION

INTRODUCTION

Year 2003 celebrated the 50th anniversary of the development of cardiopulmonary bypass (CPB) technology that has been one of the greatest advancements in cardiovascular surgery, and also established the technical background of heart and lung transplantation.

However, routine cardiac operations involving cardio-pulmonary bypass are often associated with postoperative complications related to blood contact with foreign surface and resultant activation of blood leukocytes and pro-inflammatory cytokines collectively termed systemic inflammatory response (SIRS). Cytokines play a critical role as signalling molecules that set off, intensify, and terminate local and systemic inflammatory responses. Among these proteins, tumour necrosis factor (TNFα) and interleukin-1 (IL-1β) are particularly important since they can be stimulated by a broad spectrum of stimuli, and are able to act on a large number of effectors.

According to numerous studies, specific and non-specific cytokine antagonists have also been described in the plasma of patients undergoing coronary surgery providing potential mechanisms for limiting the biologic effects of pro-inflammatory cytokines. Soluble TNF receptors bind TNF in the plasma and thus preclude it from binding to its transmembranous receptors, while IL-1 receptor antagonists occupies IL-1 receptors without any signal-transduction effect.

The vascular endothelium is known to be one of the main targets of postoperative ischaemia-reperfusion injuries, coagulopathies or systemic inflammatory responses. On the other hand, acting as a mediator organ it alters the postoperative outcome of cardiac patients through inducible genes and enzymes located in the endothelium.

Nitric Oxide (NO) plays an important role in both the physiological control of lung function and in the pathophysiology of several lung diseases. Pulmonary vascular endothelial cells and airway epithelial cells continuously generate NO from the terminal guanidino group of the amino acid L-arginine, the physiological precursor of NO by the action of NO synthases. In the lung NO acts both directly and through the soluble guanyl cyclase – cyclic GMP pathway. As endogenous production of NO can be detected and monitored in the exhaled air, it has become a valuable diagnostic and monitoring tool in chronic lung pathologies.

It is widely accepted that two prominent cytokine- responsible pathways are the inducible NO and HO-CO system. These pathways are up regulated with increased generation of the products of these inducible enzymes in both systemic and local pulmonary inflammation. Thus, exhaled NO is increased in models of septic shock and in critically ill patients with ventilator associated pulmonary infections.

Out of three known isoforms of heme oxygenase enzymes HO-1 is inducible by a broad spectrum of stimuli like hypoxia, hyperoxia, cytokines, endotoxins or UV-radiation. Similarly to NO, CO in low concentrations (bellow 0.01%) acts through the soluble guanyl cyclase – cyclic GMP pathway regulating the vasomotor tone, and has anti-inflammatory properties. Exhaled CO has also been suggested as marker of pulmonary inflammation in critically ill patients.

AIMS

1. Measuring the levels of pro- and anti-inflammatory cytokines in patients' plasma before and after routine cardiac surgery involving cardiopulmonary bypass. Establishing the net inflammatory balance represented by the relationships between ratios of pro- and anti-inflammatory mediators and their physiological significance in contributing to systemic vasodilatation and pulmonary dysfunction routinely associated with cardiac surgery.

2. Our aim was to clarify the influence and final physiological consequences of the exact cytokine balance or imbalance associated with cardiac surgery in human vascular endothelial by utilising endothelial cell-based in vitro bioassays where endothelial cell activation and apoptosis could be monitored in response to plasma from patients undergoing coronary operations.

3. Vast amounts of data indicate that pro-inflammatory conditions effectively up regulate the inducible NO and heme oxygenase (HO) systems leading to increased production of NO and carbon monoxide (CO), respectively. On the basis of recent recognition of measurement techniques and diagnostic acceptance of these molecules in the exhaled breath as measure of pulmonary inflammation, we reasoned that monitoring exhaled NO and CO in patients undergoing open heart surgery would be another way to evaluate in vivo consequences of net cytokine imbalance.

CYTOKINE BALANCE IN PATIENTS UNDERGOING ROUTINE CARDIAC SURGERY UTILISING CARDIO-PULMONARY BYPASS

Methods

40 ml venous blood was collected from each patient pre CPB following placement of central venous catheter (control) and 6 hr after initiation of CPB.

To study whether plastic "foreign" surface can result in increased cytokine activity on it's own one aliquot of blood from each blood sample was incubated for 6 hrs at 37^0C in a sterile plastic tube. As a positive control for surgery/CPB-induced inflammatory response, aliquots of blood were spiked with increasing concentrations of LPS (1, 10, 100, 1000 ng/ml) and were incubated for 6 hrs at 37^0C, prior to obtaining LPS-stimulated plasmas.

Individual cytokines and their respective modulators were assayed in patient plasma using the specific quantitative enzyme immunoassay ELISA for IL-1β, IL-1ra, TNFα or sTNF RI and RII.

Results

<u>TNFα</u>

TNFα levels at baseline were below the sensitivity of the immunoassay in the majority of the patients. TNFα levels did not increase in all but two patients 6 hours after CPB to the detectable range. When pre and post CPB blood samples were incubated for an additional 6 hours in vitro, interestingly, this further incubation increased TNFα levels about 10 times. Inclusion of LPS (1ng/ml) produced a tremendous further increase in immunogenic TNFα in pre CPB whole blood. This stimulatory effect of LPS was less when post CPB (obtained 6 hours after CPB) whole blood was incubated with LPS in vitro ($p<0.05$).

<u>IL-1β</u>

Similarly to TNFα, IL-1β levels were detectable only in a minority of patients prior to surgery and CPB, and as a group did not show any significant increase after

CPB. Similarly to TNFα, the 6-hour in vitro incubation significantly elevated IL-1β levels both in the pre and to a lesser degree in the post CPB blood even in the absence of LPS. LPS stimulation produced more than hundred fold increase in IL-1β. Treatment of post CPB blood with LPS under identical conditions increased IL-1β levels to a lesser degree ($p<0.05$).

Soluble TNF receptors

Levels of the sTNF RI and sTNF RII were detectable in the nanogram range in all patient, which increased significantly 6 hours after CPB ($p<0.05$). Further incubation of any of the patient's whole blood either in the presence or absence of LPS did not appear to change sTNF RI and sTNF RII receptor levels.

Receptor antagonists

IL-1ra levels after CPB were 10-90 fold higher than their respective pre CPB value in nearly all individual patients. Treatment of pre CPB whole blood with LPS significantly increased IL-1ra levels about ten fold. LPS also had a small incremental effect in increasing IL-1ra levels in the post CPB which already contained high levels of this protein.

sTNF RI : TNFα és sTNF RII : TNFα molar ratios

Molar plasma concentrations of both sTNF RI and II were at least two orders of magnitude higher than corresponding concentrations of TNF providing a median ratio of 449 for sTNF RI/TNF and 465 for sTNF RII/TNF. These molar ratios increased further 6 hours after CPB due to increase in sTNF RI and RII over TNF. These ratios decreased following in vitro incubation of pre CPB whole blood even in the absence of LPS because the increase in TNF was more prominent than the increase in sTNF RI and RII. LPS activation dramatically reduced these ratios producing a situation where concentrations of TNF now exceeded the concentration of sTNF RI and RII by an order of magnitude. Similar changes were seen in the post CPB blood, although the effects of in vitro incubations were less pronounced.

IL-1ra : IL-1β molar ratios

Analyses of the molar ratios reveals a 350 fold pre-dominance of IL-1ra over IL-1β before CPB. This ratio increased tremendously following CPB although individual patients exhibited more than an order of magnitude variability. In sharp contrast to the in vivo data, the whole blood in vitro assay produced decreases in these ratios due to predominant stimulated release of IL-1β over IL-1ra. In the presence of LPS, this ratio decreased to 5 and 36 in the pre and post CPB samples, respectively ($p<0.05$)

New observations

1. We demonstrated that the patients present for cardiac surgery with huge molar excess of anti-inflammatory cytokines. On the basis of these calculations there were more than 800 circulating sTNF RI and sTNF RII molecules for every molecule of TNF. Similarly to the TNF pathway, there was a 400 fold molar excess of IL-1ra over IL-1β. These observations suggest that the plasma of patients presented for open-heart surgery is equipped with a formidable repertoire to attenuate pro-inflammatory cytokine action, representing a tremendous physiologic reserve that might have the capacity to prevent widespread organ damage after heart operations.

2. Our data do not support the hypothesis that surgery/CPB up regulates the early response pro-inflammatory cytokines creating a pro-inflammatory imbalance in the plasma. Instead, our results substantiate previous observations regarding anti-inflammatory response to coronary surgery and/or CPB, involving soluble inhibitors of TNF and the soluble receptor antagonist of IL-1. In contrast to anti-inflammatory events, there was no evidence for statistically significant increases in either TNF or IL-1 concentrations at any time points following CPB when compared to pre-surgery levels.

3. In contrast to our in vivo results under our experimental conditions (6 hours incubation at 37^{o} C in sterile plastic tube) we have found consistent increases in both IL-1 and TNF concentrations causing reduced anti/pro-inflammatory ratios when compared to levels prior to these incubations in the native plasma. Thus, prolonged exposure of the stagnant whole blood to foreign plastic surface readily activated blood

cells to release these cytokines under the conditions of the experiment. In response to LPS the observed increase of IL-1 and TNF release was significantly less in the postoperative samples (endotoxin tolerance).

INFLUENCE OF PRO-INFLAMMATORY CYTOKINES AND PATIENT'S PLASMA ON HUMAN VASCULAR ENDOTHELIAL CELL DEATH

In this section we compared the influence of TNFα and IL-1β cytokines and bacterial LPS to patient's native and LPS-induced plasma before and after CPB on human endothelial cells in culture. Our major aim was to elucidate whether routine cardiac surgery involving CPB causes any imbalance of pro/anti-inflammatory and pro-apoptotic/cytoprotective bioactivities in the blood of patients that alters the viability of human vascular endothelial cells.

Methods

Human aortic (HAEC) or pulmonary artery endothelial cells (PAEC) were isolated from the aortic or pulmonary artery trimmings from heart and heart/lung donors. For experiments cells were seeded into 24 well plates and allowed to grow to 80% confluence. Endothelial cells were identified by their characteristic monolayer appearance ad by immunocytochemical staining.

Apoptosis bioassay

In order to assess the direct influence of cytokines and LPS on endothelial cell morphology and viability, human aortic endothelial cells were treated in the absence or presence of cycloheximide (10 μM) with increasing concentrations of TNF, IL- 1B and LPS for 6 hours. The effects of single cytokines were compared to native patient plasma obtained before and after CPB or to serial dilutions of plasma of LPS stimulated whole blood obtained before and after CPB. At the end of the incubation period cells were assessed for morphological criteria of apoptosis using phase contrast microscopy and for viability using the MTT assay. The role of apoptosis in endothelial cell injury was tested by investigating the role of endogenous caspases in mediating decreased viability. This was achieved by incubating the cells in the absence or presence of the cell-permeable peptide, z-VAD, a highly specific caspase inhibitor and by assessment of its influence on cytokine- and plasma-induced cell death.

Results

Cytokine-induced endothelial apoptosis in sensitised cells

In the absence of cycloheximide, there was no obvious morphological change observed in human aortic endothelial cells treated with TNF, IL-1 and LPS and there was no change in cell viability as assessed by the MTT assay. In the presence of CHX, the TNF induced cell activation and induction of cell death was unmasked, showing a concentration-dependent inhibition in cell viability over 6 hours. Similar concentration-dependent decreases in cell viability and morphological changes were observed with IL-1. LPS only caused cytotoxicity at concentrations higher that 10 ng/ml. The decrease in viability and apoptotic morphology exhibited by the endothelial cells was inhibited by ZVAD.

Influence of patient plasma samples on HAEC apoptosis

There was a small and variable degree of reduction in HAEC viability in response to post CPB plasma when compared to the appropriate pre CPB plasma samples. In contrast, significant bioactivity to cause endothelial cell death was observed in the plasmas obtained from the LPS-stimulated whole bloods. This activity was present even at 1:100 fold dilutions of the LPS-stimulated plasmas. Again, ZVAD completely prevented HAEC cytotoxicity induced by all LPS-activated plasma samples.

Finally, we compared the influence of LPS on the pre and post CPB blood samples. Although LPS produced a concentration dependent cytotoxic activity in both the pre- and post-CPB whole blood, cytotoxicity induced by the post CPB plasmas was significantly less at all LPS concentrations.

New observations

1. Using human endothelial cells in vitro bioassay we failed to demonstrate severe cytotoxic activity in patients' plasma after routine cardiac surgery utilizing CPB. On the other hand, concentration-dependent cytotoxic/apoptotic activity induced by bacterial LPS was significantly less in the post CPB plasmas than in the preoperative samples.

BASAL AND NITROGLYCERIN-INDUCED EXHALED NITRIC OXIDE IN OPEN-HEART CARDIAC SURGERY

Nitric Oxide (NO) plays an important role in both the physiological control of lung function and in the pathophysiology of several lung diseases.

As endogenous production of NO can be detected and monitored in the exhaled air it has become a valuable diagnostic and monitoring tool in acut and chronic lung pathologies. Previous studies suggest that expired NO is mainly of airway epithelial rather than of vascular endothelial origin, what would mean that altered pulmonary microvascular function might not be reflected by a change in expired NO.

However, endogenous NO pathways can be augmented by administration of NO donors, such as nitroglycerin (GTN), which elicits its biological effect by NO release mediated by thiol-dependent enzymatic biotransformation in the pulmonary microvasculature. NO then diffuses into the alveolar space, giving measurable rise to exhaled NO. On the basis of these considerations, GTN-induced exhaled NO might be a useful tool to monitor metabolic function of pulmonary microvasculature.

The aim of this study was to clarify the characteristics of NO in the expired air in the setting of routine open-heart surgery utilizing CPB. In addition we contrasted airway epithelial mechanisms (reflected as basal exhaled NO) to vascular events (judged by exhaled NO responses following intravenous administration of GTN).

Methods

Breath to breath measurements of NO concentrations in the lower airways were performed using a real-time, computer-controlled and integrated system (LR2000 series, Logan Research Ltd.). Inspired and expired samples for analysis of NO, CO and CO_2 were continuously withdrawn directly from the main lower airways through a thin Teflon sampling tube. Since detected concentration of exhaled gases depends on both the production rate and ventilation parameters, ventilation was standardized for inspired gas (100% O_2), tidal volume (5 ml/kg), respiratory rate (10/min), inspiratory and expiratory ratio (1:3) and PEEP set to zero.

Baseline measurements were performed prior to CPB to evaluate endogenous levels of exhaled NO. After the baseline measurements, three increasing boluses of 1,

2 and 3 μg/kg GTN were administered to the patient via the central venous catheter with exhaled NO response recorded. The similar protocol was repeated 1and 3 hours after CPB.

Results

In all patients NO was detectable in the exhaled air before CPB as a characteristic oscillating signal, which appeared to increase with expiration as judged by the CO_2.

Basal (endogenous) exhaled NO levels and exhaled CO levels remained unchanged 1 and 3 hours after CPB.

Intravenous bolus administration of 1, 2 and 3 μg/kg GTN resulted in a rapid, transient and dose-dependent increase in exhaled levels of NO. The time between administration of GTN bolus and detectable rise in exhaled NO was about 10-12 seconds and lasted for only a couple of breathe cycles.

There were characteristic changes in GTN-induced response in exhaled NO after CPB. The dose-dependent increases in exhaled NO by GTN were significantly smaller at 1 hour and 3 hours after CPB when compared to levels measured before CPB. As an internal control, we analysed the CO_2 output data to ensure that the changes regarding GTN-induced exhaled NO post CPB was not due to changes in exhalation profiles. This analysis reveals that baseline peak exhaled CO_2 was similar before, 1 and 3 hours after CPB and similarly, end tidal CO_2 remained the same for each respective GTN boluses before and after CPB.

New observations

1. While basal NO production mainly of epithelial origin remained unchanged in the perioperative period, our results indicate attenuation of the metabolic activity of lungs to increase exhaled NO in response to intravenous GTN following CPB, suggesting a degree of selective microvascular injury even in routine open-heart surgery.

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